Factors Impacting Persistence of Phi6 Bacteriophage, an Enveloped Virus Surrogate, on Fomite Surfaces

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ABSTRACT The persistence of Phi6 (Φ6) bacteriophage on surfaces commonly encountered in consumer-facing environments was evaluated. Φ6 has been utilized as a surrogate for enveloped viruses, including SARS-CoV-2—the causative agent of COVID-19—due to structural similarities, biosafety level 1 (BSL-1) status, and ease of use. Φ6 persistence on fomites was evaluated by characterizing the impact of the inoculum matrix (artificial saliva, phosphate-buffered saline [PBS], tripartite), inoculum level (low and high), and surface type (nonporous—aluminum, stainless steel, plastic, touchscreen, vinyl; porous—wood). Φ6 was inoculated onto surfaces at low and high inoculum levels for each inoculum matrix and incubated (20.54 ± 0.48°C) for up to 168 h. Φ6 was eluted from the surface and quantified via the double agar overlay assay to determine virus survival over time. For nonporous surfaces inoculated with artificial saliva and PBS, significantly higher *D* values were observed with high inoculum application according to the 95% confidence intervals. In artificial saliva, *D* values ranged from 1.00 to 1.35 h at a low inoculum and 4.44 to 7.05 h at a high inoculum across inoculation matrices and surfaces. *D* values for Φ6, regardless of the inoculum level, were significantly higher in tripartite than in artificial saliva and PBS for nonporous surfaces. In contrast with artificial saliva or PBS, *D* values in tripartite at low inoculum (*D* values ranging from 45.8 to 72.8 h) were greater than those at high inoculum (*D* values ranging from 26.4 to 45.5 h) on nonporous surfaces. This study characterized the impact of the inoculum matrix, inoculum level, and surface type on Φ6 survival on various surfaces relevant to fomite transmission in public settings.

IMPORTANCE An important consideration in virus contact transmission is the transfer rate between hands and surfaces, which is driven by several factors, including virus persistence on inanimate surfaces. This research characterized Φ6 persistence on surfaces commonly encountered in public settings based on various factors. The inoculum matrix, which simulates the route of transmission, can impact virus persistence, and three separate matrices were evaluated in this study to determine the impact on Φ6 persistence over time. The number of microorganisms has also been suggested to impact persistence, which was evaluated here to simulate real-world contamination scenarios on six surface types. Results from this study will guide future research utilizing Φ6 or other surrogates for enveloped viruses of public health concern.

KEYWORDS Phi6, SARS-CoV-2, carrier, inoculum matrix, persistence, surrogate

Transmission of enveloped viruses of public health importance, such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—the causative agent of COVID-19—is an important determinant to assess risk levels for future outbreaks caused by enveloped viruses. Risk assessment outcomes are guided by both direct and indirect contact transmission routes. An important consideration in virus contact transmission is the transfer rate between hands and surfaces, which is driven by several factors, including virus persistence on inanimate surfaces. In response to the COVID-19 pandemic, the stability of

SARS-CoV-2 on surfaces has been investigated on inanimate surfaces to help drive risk assessments in public health (1–5).

While it is ideal to study the pathogen of interest, e.g., SARS-CoV-2, this is not always feasible. For instance, biosafety level 3 (BSL-3) facilities are required for SARS-CoV-2 research, which is inaccessible to many researchers, cost-prohibitive, and limits the number of studies and parameters that can be investigated (6–8). These limitations highlight the utility of surrogates for assessing important experimental parameters that may not be addressed with the pathogen of interest. Surrogate selection criteria have been characterized (9), and delineating how study parameters impact surrogates can help identify their utility when studying the pathogens of interest.

Phi6 ($\Phi6$) is a segmented, double-stranded RNA bacteriophage of approximately 75 nm in diameter (10). Similar to SARS-CoV-2, $\Phi6$ bacteriophage is lipid-enveloped. $\Phi6$ infects *Pseudomonas syringae* pv. *phaseolicola*, which in addition to $\Phi6$, has BSL-1 status and can be utilized as a host (11–13). $\Phi6$ continues to be investigated as a surrogate for SARS-CoV-2 based on structural similarities of the phospholipid envelope, the relatively short analysis time (24 h), and cost-effective assays that enable experiments to be performed without specialized facilities (6, 14).

The extent of indirect contact transmission via fomites and subsequent risk of infection for SARS-CoV-2 has been controversial, as previous studies have inoculated surfaces with virus titers that do not represent real-world contamination scenarios (15, 16). Slower inactivation kinetics of viruses have been observed at higher inoculation levels (17, 18), although further evidence is needed, as inoculum matrix (e.g., artificial saliva, vomitus, feces, etc.) may impact this observation (17). Further understanding the scenarios that facilitate an increased risk of transmission due to persistence of enveloped viruses will help guide mitigation strategies in consumer-facing environments (e.g., restaurants, waiting rooms, public transportation, etc.) where fomite surfaces are commonly touched. The matrix associated with enveloped viruses has been highlighted as an important factor for persistence in the environment (19–21). Thus, evaluating how various matrices impact survival on fomites, especially in relation to virus concentration, is important to assess transmission routes driven by surface contamination scenarios.

To further substantiate $\Phi6$ as a surrogate for enveloped viruses of public health importance, persistence data based on the inoculum matrix, inoculum level, and surface type are needed. Previous studies have evaluated the persistence of $\Phi6$ on fomites (8, 17, 21–23). However, these studies have not characterized persistence on multiple unique surface types with various inoculum matrices and levels. This research was performed to characterize $\Phi6$ persistence on surfaces commonly encountered in public settings under conditions relevant to real-world exposures, including when a virus is deposited at various concentrations and within different bodily fluids (e.g., respiratory secretions, fecal material).

RESULTS AND DISCUSSION

The impact of inoculum level on virus persistence on fomites has been highlighted previously (15–17, 19). Lai et al. (19) observed greater survival of SARS-CoV-1 at higher virus titers (4, 5, and 6 log 50% tissue culture infective dose [$TCID_{50}$]/mL) with survival times ranging from <5 min to 48 h following inoculation on paper, a disposable gown, and a cotton gown. Bangiyev et al. (17) observed longer half-lives (overall range of 5 to 57 min) of $\Phi6$ in a saline inoculum matrix when vacuum-dried on plastic tubes at approximately 4 log PFU versus when evaluated at lower concentrations (3 and 2 log PFU). However, longer half-lives at increased inoculation levels were not observed with $\Phi6$ in a Luria-Bertani inoculum matrix on plastic (half-lives ranged from 9 to 18 h), which provides evidence that extended survival may not always result from an increased inoculum titer.

In the current study, a high inoculum did not consistently result in higher *D* values, as this was impacted by the inoculum matrix. For instance, artificial saliva and phosphate-

TABLE 1 Inactivation kinetics based on inoculum matrix, surface, and inoculum level[a]

Inoculum matrix	Surface	Inoculum level	D value (95% CI)	Decay rate (log PFU h^{-1}) (95% CI)	R^2
Artificial saliva	Aluminum	High	4.60 (3.41–7.05)	0.22 (0.14–0.29)	0.67
		Low	1.07 (0.80–1.63)	0.93 (0.61–1.25)	0.81
	Plastic	High	4.61 (3.58–6.46)	0.22 (0.15–0.28)	0.75
		Low	1.14 (0.83–1.81)	0.88 (0.55–1.21)	0.78
	Stainless Steel	High	4.44 (3.47–6.17)	0.23 (0.16–0.29)	0.76
		Low	1.00 (0.77–1.42)	1.00 (0.70–1.30)	0.85
	Touchscreen	High	7.05 (4.87–12.80)	0.14 (0.08–0.21)	0.55
		Low	1.16 (1.00–1.39)	0.86 (0.72–1.00)	0.95
	Vinyl	High	5.33 (4.29–7.05)	0.19 (0.14–0.23)	0.81
		Low	1.35 (0.97–2.20)	0.74 (0.45–1.03)	0.77
	Wood	High	0.77 (0.51–1.55)	1.30 (0.65–1.96)	0.66
		Low	0.61 (0.50–0.77)	1.65 (1.29–2.00)	0.96
PBS	Aluminum	High	4.63 (3.37–7.41)	0.22 (0.13–0.30)	0.67
		Low	1.49 (1.07–2.45)	0.67 (0.41–0.93)	0.76
	Plastic	High	4.30 (3.34–6.01)	0.23 (0.17–0.30)	0.75
		Low	1.49 (1.17–2.06)	0.67 (0.49–0.86)	0.87
	Stainless steel	High	2.02 (1.52–3.04)	0.49 (0.33–0.66)	0.76
		Low	0.83 (0.65–1.15)	1.21 (0.87–1.54)	0.93
	Touchscreen	High	2.54 (1.69–5.12)	0.39 (0.20–0.59)	0.61
		Low	0.79 (0.60–1.17)	1.27 (0.86–1.68)	0.86
	Vinyl	High	3.86 (3.13–5.03)	0.26 (0.20–0.32)	0.81
		Low	1.52 (1.14–2.31)	0.66 (0.43–0.88)	0.81
	Wood	High	0.50 (0.34–1.00)	1.98 (1.00–2.97)	0.80
		Low	0.72 (0.55–1.06)	1.38 (0.95–1.82)	0.95
Tripartite	Aluminum	High	26.4 (24.2–28.9)	3.79×10^{-2} (3.46–4.13×10^{-2})	0.96
		Low	72.2 (48.8–138.8)	1.39×10^{-2} (0.72–2.05×10^{-2})	0.59
	Plastic	High	36.3 (33.9–39.1)	2.75×10^{-2} (2.56–2.95×10^{-2})	0.97
		Low	62.5 (45.5–99.5)	1.60×10^{-2} (1.01–2.20×10^{-2})	0.70
	Stainless Steel	High	31.3 (28.9–34.2)	3.19×10^{-2} (2.93–3.46×10^{-2})	0.97
		Low	61.9 (45.8–95.3)	1.62×10^{-2} (1.05–2.18×10^{-2})	0.73
	Touchscreen	High	45.5 (38.7–55.3)	2.20×10^{-2} (1.81–2.58×10^{-2})	0.86
		Low	45.8 (34.9–66.5)	2.19×10^{-2} (1.50–2.87×10^{-2})	0.72
	Vinyl	High	33.1 (29.7–37.4)	3.02×10^{-2} (2.67–3.37×10^{-2})	0.94
		Low	72.8 (61.1–90.0)	1.37×10^{-2} (1.11–1.64×10^{-2})	0.90
	Wood	High	12.6 (8.4–25.4)	7.95×10^{-2} (0.04–0.12)	0.66
		Low	1.60 (1.09–2.96)	0.63 (0.34–0.92)	0.73

[a]CI, confidence interval.

buffered saline (PBS) matrices resulted in a faster decline of Φ6 at high inoculum, while the tripartite matrix resulted in a slower decline at high inoculum. In artificial saliva, D values (time to obtain one log$_{10}$ reduction) ranged from 0.61 to 7.05 h depending on the surface type and inoculum level (Table 1). At a high inoculation level, the greatest survival was observed on touchscreen with a D value of 7.05 h, and minimal differences in D values were observed among aluminum, plastic, stainless steel, and vinyl (4.44 to 5.33 h) (see Fig. S1 in the supplemental material). Minimal differences were observed among nonporous surfaces at a low inoculum level in artificial saliva (1.00 to 1.35 h) (Fig. S2). Similar to Φ6 in artificial saliva on surfaces, greater D values were observed at high (2.02 to 4.63 h) versus low inoculum levels (0.79 to 1.52 h) for all nonporous surfaces in PBS (Fig. S3 and S4).

In tripartite matrix, there was a significant difference in D values based on inoculum level for all surfaces except touchscreens according to the 95% confidence intervals. Unlike other matrices, D values in tripartite matrix at low inoculum (D values ranging from 45.8 to 72.8 h) were greater than those at high inoculum (D values ranging from 26.4 to 45.5 h) on nonporous surfaces (Fig. S5 and S6). Similar to artificial saliva, the greatest D value was observed on a touchscreen (45.5 h, high inoculum), although a similar D value was observed at a low inoculum level on a touchscreen (45.8 h). At a low inoculum level in tripartite, Φ6 on vinyl and aluminum exhibited D values greater than 72 h (Table 1), while Φ6 on plastic and stainless steel exhibited D values greater

TABLE 2 Recovery efficiency (%) and log PFU loss based on surface and inoculum matrix

Surface	Inoculum matrix[a]	Recovery efficiency (%)	Log PFU loss[b]
Aluminum	AS	64.6 ± 23.0	0.24 ± 0.24 A
	PBS	119 ± 138	0.14 ± 0.49 A
	TP	100 ± 152	0.29 ± 0.51 A
Plastic	AS	63.2 ± 23.1	0.25 ± 0.27 A
	PBS	119 ± 118	0.17 ± 0.57 A
	TP	58.4 ± 45.2	0.36 ± 0.38 A
Stainless steel	AS	58.0 ± 26.7	0.30 ± 0.29 A
	PBS	86.0 ± 82.0	0.25 ± 0.46 A
	TP	60.9 ± 48.0	0.38 ± 0.44 A
Touchscreen	AS	65.5 ± 26.0	0.22 ± 0.20 A
	PBS	49.4 ± 25.5	0.38 ± 0.31 A
	TP	48.0 ± 32.1	0.44 ± 0.37 A
Vinyl	AS	54.6 ± 26.1	0.34 ± 0.21 A
	PBS	91.4 ± 73.8	0.21 ± 0.47 A
	TP	113 ± 172	0.28 ± 0.56 A
Wood	AS	19.2 ± 20.1	1.03 ± 0.64 B
	PBS	5.87 ± 14.3	2.25 ± 1.21 B
	TP	13.2 ± 24.6	1.90 ± 1.29 B

[a]AS, artificial saliva; PBS, phosphate-buffered saline; TP, tripartite.
[b]Different letters indicate significant differences ($P < 0.05$).

than 60 h. Based on work by Bangiyev et al. (17) and the current study, the impact of the suspension medium/inoculum matrix, especially in relation to viral titer, should be considered when interpreting surrogate data in fomite persistence investigations.

The presence of organic matter is thought to represent a worst-case scenario when assessing virus inactivation (1, 24, 25). Viruses are presumed to be stabilized and protected in the presence of organic matter (20). Based on the greater survival observed at low versus high tripartite inoculum levels, further evaluation of Φ6 and its interaction with this matrix is warranted. At the low inoculum level, there may have been greater interaction of each virus with the organic matrix in comparison with a high inoculum in which greater virus-virus interaction could have resulted in less protection. Wood et al. (21) observed greater survival of Φ6 in a blood matrix than in PBS on various surfaces; median D values of Φ6 in PBS for glass and stainless steel were 23 and 5 h, respectively. However, in a blood matrix, median D values ranged from 103 to 283 h on glass and from 77 to 88 h on stainless steel. Bodily fluids may limit virus envelope damage via desiccation (6), and the protective effects of proteins have been postulated as an important factor for prolonging virus survival (18, 25–27).

The current study was performed at ambient temperatures with monitored relative humidity (RH) levels to best represent consumer-facing environments. The mean temperature in the biosafety cabinet during surface incubation was 20.54 ± 0.48°C with a maximum and minimum temperature of 23.35 and 20.13°C, respectively. A mean RH of 62.46 ± 2.64% was observed with a maximum and minimum RH of 72.29 and 47.49%, respectively. The mean inoculum level (final concentration on surfaces) for high and low inoculum levels among all inoculum matrices was 7.11 ± 0.45 and 3.33 ± 0.72 log PFU, respectively. Among all surfaces, the recovery efficiency with artificial saliva, PBS, and tripartite (consisting of bovine mucin, bovine serum albumin, and tryptone) was 54.2 ± 28.1, 74.5 ± 89.8, and 62.4 ± 94.7%, respectively. The recovery efficiencies and log PFU/mL loss at 0 h for each surface and inoculum matrix were calculated. Mean log PFU/mL loss following surface inoculation was below 0.50 log PFU/mL for each surface except wood (Table 2). There were no significant differences in the log PFU/mL loss among nonporous surfaces, regardless of the inoculum matrix type ($P > 0.05$). However, log PFU/mL loss on wood surfaces was significantly different from each nonporous surface type, regardless of inoculum matrix type ($P < 0.05$).

For wood, a low recovery of virus was observed from the surface due to the porous structure, which suggests that lower transmission risks are likely on similar porous

surfaces. Based on inoculum level, large differences in D values were observed on wood surfaces for tripartite (low, 1.60 h; high, 12.58 h), while minimal differences were observed for artificial saliva (low, 0.61 h; high, 0.77 h) and PBS (low, 0.72 h; high, 0.50 h). Among nonporous surfaces, the type of surface did not have as great of an impact on Φ6 persistence. Whitworth et al. (23) observed similar D values between stainless steel and plastic surfaces when Φ6 was suspended in an artificial test soil containing albumin proteins, hemoglobin, carbohydrates, cellulose, lipids, and salts that simulated bodily fluids. However, large differences in D values were observed for low (3.0 g/m³ [18°C, 20% RH]) and high (14.4 g/m³ [26°C, 57% RH]) absolute humidities at 14 to 18 days and 6 h, respectively (23).

The current study highlights the importance of inoculum matrix and inoculum level in relation to enveloped virus persistence on fomites and provides evidence that the survival rate of Φ6 was similar among the nonporous surface types investigated. The data generated from this study will build upon previous studies focused on utilizing the Φ6 bacteriophage as a surrogate for enveloped viruses (8, 17, 21–23). The decay rate (log PFU h^{-1}) was included in this study for previous and future comparisons of Φ6 persistence on surfaces. Overall, the lowest decay rates were observed in the tripartite matrix (1.37×10^{-2} to 3.79×10^{-2} log PFU h^{-1}), which are similar to those from a previous investigation by Whitworth et al. (23) (2.5×10^{-3} to 5.92×10^{-2} log PFU h^{-1}). Much higher decay rates were observed in PBS and artificial saliva (0.22 to 1.98 log PFU h^{-1}) in this study.

Riddell et al. (3) observed D values ranging from 33 to 42 h at 30°C and 143 to 152 h at 20°C for SARS-CoV-2 inoculated on stainless steel, glass, and vinyl surfaces with tripartite matrix. While more data are needed directly comparing different enveloped viruses simultaneously, the observed D values for Φ6 in the current study in tripartite are similar to the values observed by Riddell et al. (3) and warrant further investigation. Additionally, determining how inoculum matrix impacts inactivation kinetics for enveloped viruses will be necessary when making comparisons across persistence data sets. Factors such as protein binding sites, sources of inoculum matrix components, and virus-virus interactions, or microbial interactions in general, in the inoculum are important areas that remain to be investigated.

MATERIALS AND METHODS

Φ6 production. Φ6 bacteriophage (HER102) stock production and *Pseudomonas syringae* pv. *phaseolicola* (HER1102) growth was performed as previously described (28). Briefly, medium used for Φ6 propagation and *P. syringae* pv. *phaseolicola* growth was lysogeny (LC) broth (10 g NaCl, 10 g tryptone, 5 g yeast extract/L ultrapure water, pH adjusted to 7.5). Φ6 stock was produced by adding *P. syringae* pv. *phaseolicola* bacterial host (200 μL) (approximately 9 log CFU/mL) and 100 μL of undiluted Φ6 (approximately 10 log PFU/mL) to 5 mL of LC soft agar, and soft agar was poured onto LC agar plates via the double agar overlay (DAL) assay (29), after which dried plates were inverted and incubated at 25°C for 20 to 24 h. Φ6 was harvested from lacy-webbed plates with a 25-cm cell scraper (VWR, Radnor, PA), and following centrifugation (10 min at 3,000 × g, 4°C) supernatant was passed through a 0.45-μm sterile polyethersulfone syringe filter (Whatman, Buckinghamshire, UK) and stored at 4°C until use.

Inoculum preparation and surface inoculation. Low and high inoculum levels were developed in three separate inoculum matrices at a target concentration of 3.5 and 7.0 log PFU on surfaces, respectively. Sterile 1× phosphate-buffered saline (PBS), pH 7.4, was prepared by adding 100 μL of diluted Φ6 stock (high, 10-fold dilution; low, 1,000-fold dilution) to 5 mL of PBS. Similarly, 100 μL of diluted Φ6 stock (high, 10-fold dilution; low, 1,000-fold dilution) was added to 5 mL of artificial saliva consisting of 1.54 mM KH$_2$PO$_4$ (Sigma-Aldrich), 2.46 mM K$_2$HPO$_4$ (Fisher Scientific, Loughborough, UK), 0.04 mg/L MgCl$_2$·7H$_2$O (Alfa Aesar, Ward Hill, MA), 0.11 g/L NH$_4$Cl (VWR), 0.12 g/L (NH$_2$)$_2$CO (VWR), 0.13 g/L CaCl$_2$ (VWR), 0.19 g/L KSCN (Acros Organics, Carlsbad, CA), 0.42 g/L NaHCO$_3$ (Fisher Scientific), 0.88 g/L NaCl (VWR), 1.04 g/L KCl (VWR), and 3 g/L mucin (Sigma-Aldrich) at pH 7 (30, 31). Lastly, the tripartite matrix (5 mL) was prepared as described in international standard ASTM E2197-17 (24) by combining 3.4 mL of PBS containing Φ6 stock (low and high) with a 1.6-mL solution consisting of 0.8 mg/mL bovine mucin (Sigma-Aldrich, St. Louis, MO), 2.5 mg/mL bovine serum albumin (VWR), and 3.5 mg/mL tryptone (VWR) to mimic fluids shed by infected individuals (1, 3, 24, 32).

Surface source, preparation, and inoculation. Surfaces were purchased from a variety of sources (Table 3). Surfaces were cut to 5 by 5-cm (25-cm²) coupons similar to in previous studies (4, 33). Wood boards were purchased at 6 by 60 cm and were cut to 6 by 5-cm carriers.

Aluminum, plastic, and stainless-steel surfaces were sprayed with 70% ethanol until saturation and held for 20 min or until air-dried. Surfaces were then washed with hot, soapy water, thoroughly rinsed

TABLE 3 Surface details and sources investigated in this study

Surface	Detail(s)	Source
Aluminum	3003 grade, unpolished (mill) finish	Rose Metal Products, Inc., Springfield, MO
Plastic	Polyester	ePlastics, San Diego, CA
Stainless steel	304 grade, unpolished (mill) finish	Rose Metal Products, Inc., Springfield, MO
Touchscreen	Alkali-aluminosilicate thin sheet glass[a]	Corning Gorilla Glass, Corning, NY
Vinyl	Product code FT-029[b]	Mayer Fabrics, Indianapolis, IN
Wood	Pine, item no. 50249	Lowe's, Mooresville, NC

[a]Tempered glass (Gorilla Glass 3) with Native Damage Resistance.
[b]86% Vinyl-phthalate free face, 14% polyester backing.

with deionized (DI) water, and dried completely. Stainless-steel, aluminum, and touchscreen surfaces were wrapped in aluminum foil and steam sterilized at 121°C, 15 lb/in^2, for 30 min. Plastic, wood, and vinyl carriers were placed in a biological safety cabinet and exposed to UV light for at least 30 min as an initial decontamination procedure. Prior to inoculation, each surface type was transferred to a petri dish, placed in a biological safety cabinet, and exposed to UV light for at least 30 min.

Next, 50 μL of virus inoculum was spot-inoculated (10 $\pm$ 2 droplets) on the center of each surface. Prior to each experiment, the titers of inoculum matrices were measured to determine the inoculum levels deposited on each surface by performing 10-fold serial dilutions of the inoculum in sterile 1$\times$ PBS, and *P. syringae* pv. *phaseolicola* host (200 μL) and Φ6 dilutions (100 μL) were added to LC soft agar and plated in duplicate via the DAL assay as previously described. For negative-control surfaces, inoculum matrices without Φ6 were spot-inoculated as previously described and sampled and plated to confirm virus absence.

Environmental conditions and Φ6 elution from surfaces. Inoculated surfaces were incubated in petri dishes (without lids) in a biosafety cabinet without airflow until sampling (Fig. S7). The temperature and relative humidity were continuously monitored with a HOBO Bluetooth low-energy temperature/relative humidity data logger (Onset Computer Corporation, Bourne, MA). Destructive sampling was performed in duplicate, and time points (0, 2, 4, 12, 24, 48, 72, and 168 h) were selected based on preliminary trials of survival influenced by inoculum matrix, inoculum level, and surface type. Φ6 was eluted from each surface with 2 mL of LC broth by repeated pipetting (5 times total), after which recovered eluent was transferred to a sterile 2-mL microcentrifuge tube. Samples were serially diluted and plated in duplicate by adding 0.1 mL of sample to 0.25 mL of host in LC soft agar and plating via the DAL method in duplicate. When samples approached the limit of detection (LOD), 0.5 mL of undiluted eluent was plated in duplicate. The LOD ranged from 0.15 to 0.26 log PFU, which was influenced by the recovered eluent volume from surfaces.

Statistical analysis. Each surface was sampled in technical duplicates with two experimental trials for each time point with duplicate plating. PFU values were $\log_{10}$ transformed prior to statistical analysis. Raw PFU recovery values were divided by the inoculum deposited on the surface and multiplied by 100 to obtain percentage recovery efficiency values. Log PFU/mL recovery values were subtracted from the log PFU/mL inoculum deposited on the surface to determine the log PFU/mL loss during recovery from surfaces. One-way analysis of variance was performed to compare the log PFU/mL loss based on surface type for each inoculum matrix ($\alpha = 0.05$). Mean values were compared with Tukey's honestly significant difference test ($\alpha = 0.05$). Samples below the LOD were assigned a value of 0 log PFU. The log reduction of Φ6 at specific time points was calculated by subtracting the starting log PFU concentration deposited on surfaces from the log PFU concentration recovered from surfaces. Least-squares regression methods were used to fit linear models to each surface treatment for Φ6 concentrations recovered between 0 h and the first sampling time approaching the LOD. Outlier values, caused by unusually low Φ6 recovery from surfaces, were omitted from linear models. These outliers exhibited either low recovery at 0 h ($<$1 log PFU) or low recovery at other sampling times where subsequent samples resulted in a difference of $>$3 log PFU. Additionally, in the low-inoculum level with tripartite, the 24-h samples for aluminum, plastic, touchscreen, stainless steel, and vinyl were excluded from linear regression analysis due to high variability (standard deviations of 0.69 to 1.28) compared to the other sampling times (standard deviations of 0.02 to 0.50). Linear models determined the decay rate (log PFU h^{-1}) for Φ6 on each surface, and R^2 was used to assess goodness-of-fit. D values were calculated as the negative reciprocal of the decay rate (slope) for each linear model. All statistical analyses were performed in R version 4.1.1 (http://www.R-project.org).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.
SUPPLEMENTAL FILE 1, PDF file, 3 MB.

REFERENCES

1. Kalsoff SB, Leung A, Strong JE, Funk D, Cutts T. 2021. Stability of SARS-CoV-2 on critical personal protective equipment. Sci Rep 11:984. https://doi.org/10.1038/s41598-020-80098-3.

2. Paton S, Spencer A, Garratt I, Thompson K-A, Dinesh I, Aranega-Bou P, Stevenson D, Clark S, Dunning J, Bennett A, Pottage T. 2021. Persistence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus and viral RNA in relation to surface type and contamination concentration. Appl Environ Microbiol 87:e00526-21. https://doi.org/10.1128/AEM.00526-21.

3. Riddell S, Goldie S, Hill A, Eagles D, Drew TW. 2020. The effect of temperature on persistence of SARS-CoV-2 on common surfaces. Virol J 17:145. https://doi.org/10.1186/s12985-020-01418-7.

4. Ronca SE, Sturdivant RX, Barr KL, Harris D. 2021. SARS-CoV-2 viability on 16 common indoor surface finish materials. HERD 14:49–64. https://doi.org/10.1177/1937586721991535.

5. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, Tamin A, Harcourt JL, Thornburg NJ, Gerber SI, Lloyd-Smith JO, de Wit E, Munster VJ. 2020. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med 382:1564–1567. https://doi.org/10.1056/NEJMc2004973.

6. Aquino de Carvalho N, Stachler EN, Cimabue N, Bibby K. 2017. Evaluation of Phi6 persistence and suitability as an enveloped virus surrogate. Environ Sci Technol 51:8692–8700. https://doi.org/10.1021/acs.est.7b01296.

7. Castaño N, Cordts SC, Kurosu Jalil M, Zhang KS, Koppaka S, Bick AD, Paul R, Tang SKY. 2021. Fomite transmission, physicochemical origin of virus−surface interactions, and disinfection strategies for enveloped viruses with applications to SARS-CoV-2. ACS Omega 6:6509–6527. https://doi.org/10.1021/acsomega.0c06335.

8. Gallandat K, Lantagne D. 2017. Selection of a biosafety level 1 (BSL-1) surrogate to evaluate surface disinfection efficacy in Ebola outbreaks: comparison of four bacteriophages. PLoS One 12:e0177943. https://doi.org/10.1371/journal.pone.0177943.

9. Sinclair RG, Rose JB, Hashsham SA, Gerba CP, Haas CN. 2012. Criteria for selection of surrogates used to study the fate and control of pathogens in the environment. Appl Environ Microbiol 78:1969–1977. https://doi.org/10.1128/AEM.06582-11.

10. Gonzalez CF, Langenberg WG, Van Etten JL, Vidaver AK. 1977. Ultrastructure of bacteriophage φ6: arrangement of the double-stranded RNA and envelope. J Gen Virol 35:353–359. https://doi.org/10.1099/0022-1317-35-2-353.

11. Prussin IIA, Schwake DO, Lin K, Gallagher DL, Buttling L, Marr LC. 2018. Survival of the enveloped virus Phi6 in droplets as a function of relative humidity, absolute humidity, and temperature. Appl Environ Microbiol 84:e00551-18. https://doi.org/10.1128/AEM.00551-18.

12. Turgeon N, Toulouse M-J, Martel B, Moineau S, Duchaine C. 2014. Comparison of five bacteriophages as models for viral aerosol studies. Appl Environ Microbiol 80:4242–4250. https://doi.org/10.1128/AEM.00767-14.

13. Vidaver AK, Koski RK, Van Etten JL. 1973. Bacteriophage Φ6: a lipid-containing virus of *Pseudomonas phaseolicola*. J Virol 11:799–805. https://doi.org/10.1128/JVI.11.5.799-805.1973.

14. Adcock NJ, Rice EW, Sivaganesan M, Brown JD, Stallknecht DE, Swayne DE. 2009. The use of bacteriophages of the family *Cystoviridae* as surrogates for H5N1 highly pathogenic avian influenza viruses in persistence and inactivation studies. J Environ Sci Health A Tox Hazard Subst Environ Eng 44:1362–1366. https://doi.org/10.1080/10934520903217054.

15. Goldman E. 2020. Exaggerated risk of transmission of COVID-19 by fomites. Lancet Infect Dis 20:892–893. https://doi.org/10.1016/S1473-3099(20)30561-2.

16. Goldman E. 2021. SARS Wars: the fomites strike back. Appl Environ Microbiol 87:e00653-21. https://doi.org/10.1128/AEM.00653-21.

17. Bangiyev R, Chudaev M, Schaffner DW, Goldman E. 2021. Higher concentrations of bacterial enveloped virus Phi6 can protect the virus from environmental decay. Appl Environ Microbiol 87:e01371-21. https://doi.org/10.1128/AEM.01371-21.

18. Lin K, Schulte CR, Marr LC. 2020. Survival of MS2 and Φ6 viruses in droplets as a function of relative humidity, pH, and salt, protein, and surfactant concentrations. PLoS One 15:e0243505. https://doi.org/10.1371/journal.pone.0243505.

19. Lai MY, Cheng PK, Lim WW. 2005. Survival of severe acute respiratory syndrome coronavirus. Clin Infect Dis 41:e67–e71. https://doi.org/10.1086/433186.

20. Vasickova P, Pavlik I, Verani M, Carducci A. 2010. Issues concerning survival of viruses on surfaces. Food Environ Virol 2:24–34. https://doi.org/10.1007/s12560-010-9025-6.

21. Wood JP, Richter W, Sunderman M, Calfee MW, Serre S, Mickelsen L. 2020. Evaluating the environmental persistence and inactivation of MS2 bacteriophage and the presumed Ebola Virus surrogate Phi6 using low concentration hydrogen peroxide. Environ Sci Technol 54:3581–3590. https://doi.org/10.1021/acs.est.9b06034.

22. Fedorenko A, Grinberg M, Orevi T, Kashtan N. 2020. Survival of the enveloped bacteriophage Phi6 (a surrogate for SARS-CoV-2) in evaporated saliva microdroplets deposited on glass surfaces. Sci Rep 10:22419. https://doi.org/10.1038/s41598-020-79625-z.

23. Whitworth C, Mu Y, Houston H, Martinez-Smith M, Noble-Wang J, Coulliette-Salmond A, Rose L. 2020. Persistence of bacteriophage Phi 6 on porous and nonporous surfaces and the potential for Its use as an Ebola virus or coronavirus surrogate. Appl Environ Microbiol 86:e01482-20. https://doi.org/10.1128/AEM.01482-20.

24. ASTM International. 2017. ASTM E2197-17: standard quantitative disk carrier test method for determining bactericidal, virucidal, fungicidal, mycobactericidal, and sporicidal activities of chemicals. ASTM International, West Conshohocken, PA.

25. Thompson SS, Flury M, Yates MV, Jury WA. 1998. Role of the air-water-solid interface in bacteriophage sorption experiments. Appl Environ Microbiol 64:304–309. https://doi.org/10.1128/AEM.64.1.304-309.1998.

26. Thompson SS, Yates MV. 1999. Bacteriophage inactivation at the air-water-solid interface in dynamic batch systems. Appl Environ Microbiol 65:1186–1190. https://doi.org/10.1128/AEM.65.3.1186-1190.1999.

27. Yang W, Elankumaran S, Marr LC. 2012. Relationship between humidity and Influenza A viability in droplets and implications for Influenza's seasonality. PLoS One 7:e46789. https://doi.org/10.1371/journal.pone.0046789.

28. Baker CA, Gibson KE. 2022. Phi 6 recovery from inoculated fingerpads based on elution buffer and methodology. J Virol Methods 299:114307. https://doi.org/10.1016/j.jviromet.2021.114307.

29. Kropinski AM, Mazzocco A, Waddell TE, Lingohr E, Johnson RP. 2009. Enumeration of bacteriophages by double agar overlay plaque assay, p 69–76. *In* Clokie MRJ, Kropinski AM (ed), Bacteriophages: methods and protocols, vol 1. Isolation, characterization, and interactions. Humana Press, Totowa, NJ.

30. ASTM International. 2016. ASTM E2721-16: standard practice for evaluation of effectiveness of decontamination procedures for surfaces when

challenged with droplets containing human pathogenic viruses. ASTM International, West Conshohocken, PA.

31. Owen L, Shivkumar M, Laird K. 2021. The stability of model human coronaviruses on textiles in the environment and during health care laundering. mSphere 6:e00316-21. https://doi.org/10.1128/mSphere.00316-21.

32. Sattar S, Springthorpe V, Adegbunrin O, Zafer A, Busa M. 2003. A disc-based quantitative carrier test method to assess the virucidal activity of chemical germicides. J Virol Methods 112:3–12. https://doi.org/10.1016/S0166-0934(03)00192-7.

33. Buckley D, Dharmasena M, Fraser A, Pettigrew C, Anderson J, Jiang X. 2018. Efficacy of silver dihydrogen citrate and steam vapor against a human norovirus surrogate, feline calicivirus, in suspension, on glass, and on carpet. Appl Environ Microbiol 84:e00233-18. https://doi.org/10.1128/AEM.00233-18.